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Evaluation of the API-ZYM system for identification of *Listeria*

The API-ZYM system (Analytab Products) was evaluated for potential use for confirming the identity of Listeria *isolates. A total of 68* Listeria *strains from various sources were assayed using standard procedures. All isolates were positive for esterase (C4), esterase lipase (C8), phosphohydrolase (one exception), β-glucosidase, acid phosphatase and lipase (one exception). Negative responses were observed consistently for valine aminopeptidase, trypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-fucosidase and cystine aminopeptidase (one exception). Variable responses were noted for alkaline phosphatase, leucine aminopeptidase, chymotrypsin, α-glucosidase and N-acetyl glucosaminidase. Comparison of these results with those of other Gram positive species indicated that the API-ZYM system could be used for rapid confirmation of* Listeria *to the subgenus level.*

Introduction

Recent foodborne outbreaks of listeriosis have established a clear need for effective methodology for isolation and identification of *Listeria* species from food, environmental and biological materials. The need for rapid methods is particularly acute since standard protocols are time consuming and labor intensive. Several methods have been introduced including commercially available ELISA and gene probe techniques. The Micro-ID (Robinson et al. 1988) and the API-20 STREP system (Macgowan et al. 1989) have been reported capable of confirming *Listeria* to the genus level, but there has been relatively little other research into the potential application of commercially-available, miniaturized enzyme or cultural multi-test assays for the identification or confirmation of *Listeria*. Review of the API-ZYM system suggested that this product might be useful for identifying *Listeria*. Accordingly, the objectives of the current study were to characterize the response of a range of *Listeria* isolates encompassing a variety of species and sources and to determine if this system, could be used to rapidly confirm *Listeria* isolates.

Materials and Methods

Bacteria

The source and special characteristics of the *Listeria* (67), *Staphylococcus* (11), *Jonesia denitrificans* (1) and *Kurthia zopfii* (2) isolates employed are listed in Table 1. All bacteria were grown on tryptose phosphate agar (TPA)-(Difco) for 24 h at 30°C. Bacterial cultures were then resuspended in sterile saline. Each suspension was adjusted to an opacity of a MacFarland 5 standard.

Table 1. Micro-organisms examined in study.

Isolated	Identification	Source
F-4087	*J. denitrificans*	(CDC)
F-4085	*L. grayi*	(CDC)
F-4076	*L. murrayi*	(CDC)
F-4080	*L. seeligeri*	(CDC)
Type-1	*L. seeligeri*	raw beef (University of Wisconsin)
SA3-VT	*L. innocua*	salami (USDA)
GV2-VS	*L. innocua*	ground veal (USDA)
GV9-LS	*L. innocua*	ground veal (USDA)
LG5-LS	*L. innocua*	ground lamb (USDA)
SH3-VT	*L. innocua*	frozen shrimp (USDA)
P5-VS	*L. innocua*	pork sausage (USDA)
Type-4	*L. innocua*	beef fat (USDA)
SS-VJ-S	*L. innocua*	shrimp salad (USDA)
H2-L-G	*L. innocua*	hamburger (USDA)
LA-1	*L. innocua*	cheese (FDA)
CF1-VP	*L. welshimeri*	frozen chicken (USDA)
CF6-LP	*L. welshimeri*	frozen chicken (USDA)
CF6-VP	*L. welshimeri*	frozen chicken (USDA)
CF2-VP	*L. welshimeri*	frozen chicken (USDA)
H2-V-G	*L. welshimeri*	hamburger (USDA)
F-4082	*L. welshimeri*	(CDC)
CCR1-V-G	*L. welshimeri*	chicken patties (USDA)
CCR9-L-G	*L. welshimeri*	chicken patties (USDA)
CCR6-L-G	*L. welshimeri*	chicken patties (USDA)
CCR2-V-G	*L. welshimeri*	chicken patties (USDA)
GVL4-V-S	*L. welshimeri*	ground veal (USDA)
FR-101	*L. welshimeri*	meat (University of Wisconsin)
KCL-1714	*L. ivanovii*	(CDC)
F-4084	*L. ivanovii*	(CDC)
F-5999	*L. ivanovii*	(CDC)
F-6000	*L. ivanovii*	(CDC)
F-4081	*L. ivanovii*	(CDC)
P-476	*L. monocytogenes*	Hem− Tn-916 (University of Pennsylvania)
P-590	*L. monocytogenes*	Hem− Tn-916 (University of Pennsylvania)
P-433	*L. monocytogenes*	Hem− Tn-916 (University of Pennsylvania)
P-571	*L. monocytogenes*	Hem− Tn-916 (University of Pennsylvania)
P-543	*L. monocytogenes*	Hem− Tn-916 (University of Pennsylvania)
P-585	*L. monocytogenes*	Hem− Tn-916 (University of Pennsylvania)
P-570	*L. monocytogenes*	Hem− Tn-916 (University of Pennsylvania)
P-434	*L. monocytogenes*	Hem+ Tn-916 (University of Pennsylvania)
P-524	*L. monocytogenes*	Hem+ Tn-916 (University of Pennsylvania)
P-104035	*L. monocytogenes*	Hem+ (University of Pennsylvania)
15313	*L. monocytogenes*	Hem− (ATCC)
512	*L. monocytogenes*	Rh− (USDA)

Table 1. Continued.

Isolated	Identification	Source
5064	*L. monocytogenes*	Rh− (USDA)
H4-V-G	*L. monocytogenes*	hamburger (USDA)
H0-V-S	*L. monocytogenes*	hamburger (USDA)
F2-VJ-G	*L. monocytogenes*	fish (USDA)
F2-L-G	*L. monocytogenes*	fish (USDA)
MF2-L-G	*L. monocytogenes*	fish (USDA)
F3-V-G	*L. monocytogenes*	fish (USDA)
Scott-A	*L. monocytogenes*	clinical (FDA)
F-4259	*L. monocytogenes*	(CDC)
F-4561	*L. monocytogenes*	(CDC)
F-4260	*L. monocytogenes*	(CDC)
S9-V-G	*L. monocytogenes*	sausage (USDA)
S9-N-S	*L. monocytogenes*	sausage (USDA)
V3-VT	*L. monocytogenes*	veal patties (USDA)
GVS2-VJ	*L. monocytogenes*	veal patties (USDA)
GVN4-VG	*L. monocytogenes*	veal patties (USDA)
VS2-VS	*L. monocytogenes*	veal patties (USDA)
DA-1	*L. monocytogenes*	cheese (FDA)
LA-18	*L. monocytogenes*	cheese (FDA)
LA-23	*L. monocytogenes*	cheese (FDA)
LA-16	*L. monocytogenes*	cheese (FDA)
LA-7	*L. monocytogenes*	cheese curd (FDA)
CCR8-VG	*L. monocytogenes*	chicken patties (USDA)
SH6-VJ	*L. monocytogenes*	frozen shrimp (USDA)
33403	*K. zopfii*	ATCC
6900	*K. zopfii*	ATCC
196-E	*S. aureus*	(USDA)
B-Tox	*S. aureus*	(USDA)
S-1026	*S. aureus*	(University of Georgia)
S-1037	*Staphylococcus* spp.	(University of Georgia)
S-1020	*Staphylococcus* spp.	(University of Georgia)
S-1038	*S. aureus*	(University of Georgia)
S-1022	*S. aureus*	(University of Georgia)
MF2-S1	*S. aureus*	Human (USDA)
MFS-S6	*S. aureus*	Human (USDA)
MF2-S5	*Staphylococcus* spp.	Human (USDA)
MF2-S7	*Staphylococcus* spp.	Human (USDA)

API-ZYM assays

The API-ZYM assay kit (Analytab products, Plainview, NY) was used to detect 19 constituitive enzymes. Two drops of bacterial suspension were added to each of the 20 cupules per panel. Each panel was incubated in its humidification chamber at 37°C for 4 h. After incubation, 1 drop of reagent A [tris(hydroxymethyl) aminomethane, 2·5 g; HCl (37%), 1·1 ml; lauryl sulfate, 1·0 g; distilled water, 10 ml] and 1 drop of reagent B (Fast Blue BB, 35·0 mg; and 2-methoxyethanol, 10·0 ml) were added to each cupule. Color reactions were allowed to develop for 5 min, after which the cupules were exposed to a high intensity light source for 10 s. The presence and degree of enzymatic activity were scored on a 0–5 scale based on comparison against the color intensity chart provided by the manufacturer.

Results

The enzymatic activities (expressed as number of strains giving a particular

reaction) of the 66 of '*monocytogenes*-subgenus' of *Listeria* (i.e. *L. monocytogenes. L. innocua, L. welshimeri, L. seeligeri* and *L. ivanovii*) (Rocourt et al. 1987a) are summarized in Table 2. All isolates were positive for esterase, esterase lipase, phosphohydrolase (one exception), β-glucosidase, acid phosphatase and lipase (one exception), and negative for valine aminopeptidase, trypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-mannosidase, α-fucosidase and cystine aminopeptidase (one exception). Alkaline phosphatase, leucine aminopeptidase, chymotrypsin, α-glucosidase and *N*-acetyl glucosaminidase reactions varied among the isolates.

The enzymatic responses of isolates of '*murrayi-grayi* subgenus' of *Listeria* (i.e. *L. murrayi, L. grayi*) (Rocourt et al. 1987b), *Jonesia denitrificians* (previously *L. denitrificians*), *Staphylococcus* spp., and *Kurthia zopfii* were also determined (Table 3). These *Listeria* spp. differed from the '*monocytogenes*-subgenus' by their inability to produce acid phosphatase. Both *Staphylococcus* and *Kurthia* isolates differed from the '*monocytogenes*-subgenus' of *Listeria* by their lack of β-glucosidase activity.

Evaluation of replicate TPA cultures indicated that variation in quantitative enzyme responses were minimal, and there were no qualitative differences in the enzyme profiles of replicate cultures. The potential impact of using other culture media on the effectiveness of the API-ZYM assay system was assessed by comparing the enzyme profiles for eight *Listeria* isolates grown in TPA and sheep blood agar (BA). Results indicated a 17% incidence of higher quantitative enzymatic activities (i.e. greater color response in API-ZYM assay) for cells from BA cultures, whereas there was a 6% incidence of greater activity for TPA cultures over BA grown cultures. The enzyme profiles for BA and TPA cultures were qualitatively similar, though relatively minor differences were noted in certain instances. There was one occasion each when low levels of valine aminopeptidase, leucine aminopeptidase, and chymotrypsin activity were detected in BA, but not TPA grown cells. Further, there were three instances of differential detection of N-acetyl glucosaminidase activity in BA cells. Conversely, there was one instance when chymotrypsin activity was observed with TPA culture, but not with the corresponding BA culture. Non-hemolytic and rhamnose-negative *L. monocytogenes* demonstrated similar enzymatic profiles as the typical strains of *L. monocytogenes*.

Discussion

The API-ZYM system allowed *Listeria* isolates to be rapidly analyzed for 19 enzymatic activities within 4 h. In some instances cells cultured on BA gave higher enzyme activities than TPA. Additionally, some variations in the enzymes patterns of BA and TPA grown cells were observed; however, these differences were associated with enzymes that were not critical for the differentiation of *Listeria*. Accordingly, TPA was selected for routine culturing of samples to be analyzed with the API-ZYM system due to its availability, shelflife and ease of preparation.

The absence of valine aminopeptidase, trypsin, α-galactosidase, β-galactosidase, β-glucoronidase, α-mannosidase, α-fucosidase and cystine aminopeptidase (one exception), and the presence of esterase, esterase lipase, phosphohydrolase (one exception), β-glucosidase, acid phosphatase and lipase (one exception) in all 66 isolates of the '*monocytogenes*-subgenus' of *Listeria* indicated that these were general characteristics of the subgenus. Contrary to our results, Seeliger and Jones (1986) observed that all *Listeria* possessed β-galactosidase activity. This

Table 2. Enzymatic profiles of *Listeria* isolates as determined by the API-ZYM system[a].

Enzyme[a]	Percentage of isolates																				
	L. monocytogenes (28)[b]			Non-hemolytic *L. monocytogenes* (8)			*L. innocua* (10)			*L. welshimeri* (12)			*L. ivanovii* (5)			*L. seeligeri* (2)			Total (65)		
	0	1–2	3–5	0	1–2	3–5	0	1–2	3–5	0	1–2	3–5	0	1–2	3–5	0	1–2	3–5	0	1–2	3–5
Esterase	—	1[c]	27	—	—	8	—	—	10	—	—	12	—	—	5	—	—	2	—	1	64
Esterase Lipase	—	27	1	—	7	1	—	10	—	—	—	12	—	2	3	—	2	—	—	57	8
Phosphohydrolase	1	15	12	—	2	6	—	8	2	—	10	2	—	—	5	—	2	—	1	35	29
Beta-Glucosidase	—	1	27	—	—	10	—	4	6	—	3	9	—	4	1	—	—	2	—	12	53
Acid Phosphatase	—	15	13	—	5	3	—	7	3	—	10	2	—	—	5	—	—	2	—	34	31
Lipase	—	28	—	1	7	—	—	10	—	—	12	—	—	5	—	—	2	—	1	64	—
Cystine Aminopeptidase	28	—	—	7	1	—	10	—	—	12	—	—	5	—	—	2	—	—	64	1	—
Alkaline Phosphatase	7	21	—	2	6	—	—	10	—	1	11	—	—	5	—	—	2	—	10	55	—
Leucine Aminopeptidase	6	22	—	—	8	—	1	9	—	2	10	—	—	5	—	—	2	—	10	55	—
Chymotrypsin	28	—	—	7	1	—	10	—	—	12	—	—	3	2	—	1	1	—	4	61	—
Alpha-Glucosidase	2	24	2	—	7	1	3	6	1	10	2	—	4	1	—	—	2	—	19	42	4
N-Acetyl Glucosaminidase	25	3	—	8	—	—	9	1	—	12	—	—	5	—	—	2	—	—	61	4	—

[a]All *Listeria* isolates were negative for valine aminopeptidase, trypsin, α-glactosidase, β-galactosidase, β-glucuronidase, α-mannosidase and α-fucosidase.
[b]Values in parentheses indicate number of isolates examined.
[c]Number of positive isolates.

Table 3. Enzymatic profiles of *Staphylococcus, Kurthia, Jonesia* and members of the murrayi-*grayi* subgenus of *Listeria*.

	Percentage of isolates																	
	K. zopfil (2)[b]			*S. aureus* (7)			*Staphylococcus* spp. (4)			*L. murray-grayi* group (2)			*J. denitrificans* (1)		
Enzyme[a]	0	1–2	3–5	0	1–2	3–5	0	1–2	3–5	0	1–2	3–5	0	1–2	3–5
Esterase	—	2[c]	—	—	1	6	—	—	4	—	—	2	1	—	—
Esterase Lipase	—	2	—	—	1	6	—	—	4	—	2	—	1	—	—
Phosphohydrolase	—	2	—	1	6	—	4	—	—	—	2	—	—	1	—
Beta-Glucosidase	2	—	—	7	—	—	4	—	—	—	—	2	1	—	—
Acid Phosphatase	—	1	1	—	—	7	2	2	—	2	—	—	1	—	—
Lipase	—	2	—	1	6	—	—	4	—	—	2	—	1	—	—
Valine Aminopeptidase	—	2	—	7	—	—	4	—	—	2	—	—	1	—	—
Cystine Aminopeptidase	2	—	—	7	—	—	4	—	—	—	2	—	1	—	—
Alkaline Phospatase	—	2	—	—	1	6	—	2	2	2	—	—	1	—	—
Leucine Aminopeptidase	—	2	—	5	2	—	3	1	—	—	—	2	—	—	1
Chymtrypsin	1	1	—	7	—	—	4	—	—	2	—	—	1	—	—
Alpha-Glucosidase	2	—	—	2	5	—	—	3	1	1	1	—	—	—	1

[a]All cultures negative for trypsin, β-galactosidase, α-glucuronidase, α-mannosidase, α-fucosidase and *N*-acetyl glucosaminidase, α-galactosidase.
[b]Values in parentheses indicate number of isolates examined.
[c]Number of positive isolates.

difference is likely due to the lack of an inducer of β-galactosidase in the growth medium employed in the current study. In addition, our results agree for the most part with the evaluation of Roucourt and Catimel (1985) of the API-ZYM except that these investigators observed all *Listeria* to be lacking lipase activity. This discrepancy could be attributed to the low activity of this enzyme (1 in the scale of 5) observed in our study.

Streptococci, particularly enterococci, are often able to grow in media used for the isolation of *Listeria*. Previous investigations with streptococci and the API-ZYM system (Waitkins et al. 1980) have shown that enterococci lack both β-glucosidase and lipase activities, a key distinguishing characteristic between *Streptococcus* and *Listeria*. *Staphylococcus* and *Kurthia*, two other genera commonly encountered in analyses for *Listeria*, could be differentiated on the basis of β-glucosidase activity.

Virulence in *L. monocytogenes* is strongly associated with the production of hemolysin (listeriolysin O); however, it has been suggested that there may be one or more additional factors that influence the pathogenicity of the species. A number of other enzymes, including catalase, glucose dehydrogenase, lactate dehydrogenase, superoxide dismutase, lysozyme, peroxidase, lipase (phospholipase C) and lecithinase have been proposed as playing a role in virulence, though unequivocal associations have not been established (Stricker et al. 1963, Kujumgiev 1966, Elischerova and Ciznar 1970, Jenkins and Watson 1971, Ralovich et al. 1972, Khan et al. 1973, 1975, Leighton et al. 1975, Welch et al. 1979, Welch 1988). Elischerova and Ciznar (1970) and Mrsvic et al. (1975) reported that a quantitative correlation existed in *L. monocytogenes* between acid phosphatase activity and virulence to mice. The lack of differences in the enzyme profiles of hemolytic *L. monocytogenes*, and the non-pathogenic species in the '*monocytogenes*-subgenus' suggest that none of the enzymes assayed by the API-ZYM, including acid phosphatase, are related to virulence. However, definite proof will require further study. The current results with hemolytic and non-hemolytic isolates are in agreement with previous studies (Jenkins et al. 1966, Jenkins and Watson 1971) that indicated that lipase and hemolytic activities are separate entities.

In summary, members of the '*monocytogenes*-subgenus' of *Listeria* gave a characteristic enzyme pattern using the API-ZYM system. This pattern was different from other Gram-positive bacteria commonly encountered during the isolation of *Listeria*, and suggests that this system may have application as a rapid method for confirming the identity of *Listeria* isolates to the subgenus level.

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